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RESEARCH ARTICLE

Prevalence of Metallo-betalactamase Producing *Pseudomonas aeruginosa* in Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi

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Abstract

Pseudomonas aeruginosa is a common nosocomial pathogen that readily develops resistance to cephalosporins like Ceftazidime and beta-lactam antibiotics such as Imipenem through the production of Metallo-beta-lactamase (MBL). This study investigated the prevalence of MBL-producing *P. aeruginosa* at NAUTH, Nnewi. A total of 125 swab samples were collected from 44 ward door handles and 81 patient bedside handles. Samples were cultured on selective media to isolate *P. aeruginosa*, followed by standard identification using Gram staining and biochemical tests. Antibiotic sensitivity testing (AST) was performed, and Ceftazidime-resistant isolates were screened for MBL production using Imipenem alone, Imipenem with EDTA, and Imipenem with Dipicolinic acid. Contamination was highest on patient bedside handles (62.5%), followed by ward door handles (25%) and controls (12.5%); no isolates were found on laboratory door handles. The Male Surgical Ward (MSW) had the highest *P. aeruginosa* prevalence (57.14%), followed by the Children's Ward (28.57%) and Female Surgical Ward (14.29%). MBL-producing strains were most prevalent in MSW (66.67%), followed by FSW (33.33%). No MBL-producing strains were found in the Children's Ward. One ward door handle isolate (25%), two bedside isolates (50%), and one control (25%) tested positive for MBL production. Imipenem-EDTA showed the highest inhibition zone (29 mm), followed by Imipenem-Dipicolinic acid (28 mm), while Imipenem alone had the least (11 mm). Imipenem combined with EDTA was most effective against MBL-producing *P. aeruginosa*. Regular disinfection of high-touch surfaces in affected wards is strongly recommended.

Keywords: Handles, *Pseudomonas aeruginosa*, Metallo-beta lactamase, Imipenem, Imipenem with chelating agents (EDTA, Dipicolinic acid).

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1. Introduction

Pseudomonas aeruginosa is a leading cause of healthcare-associated infections (HCAIs) worldwide, accounting for approximately 10% of all HCAIs and ranking as the fourth most common nosocomial pathogen[1] It can cause a range of infections from minor skin issues to severe sepsis, with colonization in critical organs potentially being fatal[2]. Recent studies show a concerning rise in *P. aeruginosa* isolates from clinical and environmental sources, highlighting increasing nosocomial infections[3].

The World Health Organization[4] emphasizes antimicrobial resistance as a top global health threat, noting P. aeruginosa's diverse resistance mechanisms, especially enzyme production like β-lactamases, which compromise major treatment options such as β-lactam antibiotics[5]. These enzymes, particularly classes A, B, C, and D, include metallo-beta-lactamases (MBLs), which are highly problematic due to their ability to hydrolyze most β-lactams, except monobactams[6,7]. MBL-producing strains have been linked to significant nosocomial infections worldwide since their first report from Japan in 1991, spreading across Asia, Europe, Australia, South America, and North America[8], but data from Africa and Nigeria remain limited.

The emergence of MBLs contributes to high resistance rates, especially as *P. aeruginosa* can thrive in hospital environments with extensive antimicrobial use, infecting vulnerable burn patients rapidly[9]. Treating *P. aeruginosa* infections is challenging[10] because of rising antibiotic resistance, with the bacterium naturally resistant to narrow-spectrum penicillins, early-generation cephalosporins, sulfonamides, and trimethoprim[9,11]. Prevalence rates of multidrug-resistant *P. aeruginosa* vary: 87.5% in Iran, 68.8% in Malaysia, and 29.24% in Pakistan[12]. Although carbapenems are used against MDR strains, resistance is increasing worldwide due to mechanisms like loss of OprD porin, efflux pump upregulation, or MBL production, which hydrolyze carbapenems[13,14].

MBL enzymes, classified as Ambler class B, require zinc for activity and can hydrolyze various β-lactams, including penicillins, cephalosporins, and carbapenems, being inhibited by metal chelators such as EDTA[15]. These genes are often located on mobile genetic elements like plasmids and integrons, facilitating widespread dissemination among Gram-negative bacteria[16]. Five main MBL types—IMP, VIM, GIM, SIM, and Sao Paulo—are known, with VIM and IMP being predominant in P. aeruginosa[17]. Notably, IMP was initially confined to Brazilian hospitals before spreading globally, partly due to human travel[17]. Early detection of MBL-producing organisms is critical for effective treatment, especially for seriously ill and hospitalized patients, and for preventing nosocomial transmission[18]. This study aims to assess the prevalence of MBL-producing *P. aeruginosa* within specific areas of the NAUTH environment.

2. Materials and Methods

2.1 Study Design

This study is cross-sectional and aims to detect the prevalence of *Pseudomonas aeruginosa* bacteria that produce Metallo-beta-lactamase (MBL) in particular areas of NAUTH, Nnewi. The investigation was conducted between April and June of 2021.

2.2 Study Area

The investigation was conducted in six (6) major laboratory locations and twelve (12) wards at NAUTH, Nnewi.

2.3 Sample Size

As explained by Akiluet al., 2016, Daniel's sample size determination formula was used to calculate the sample size for this study. Consequently, a minimum of 125 swab samples from the various wards and laboratories involved in this study are required.

2.4 Sampling Technique:

The study used a simple random sampling approach.

2.5 Criteria for Selection

The inclusion criteria used for the study are ward, bedside, and laboratory handles of wards and laboratories in NAUTH Nnewi. The exclusion criteria used for the study are ward, bedside, and laboratory handles of wards and laboratories outside NAUTH Nnewi.

2.6 Ethical Consideration

The ethical board of Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, was consulted for ethical permission. The Matrons and Laboratory Scientists in charge of each ward and the lab were asked for their informed consent.

2.7 Sample Collection

Samples were collected by going from laboratory to laboratory and also from ward to ward, swabbing the door handles as well as bedside handles of hospitalized patients (at least a month old). The

samples were collected with sterile latex gloves, at noon during working hours, to maximize the chances of isolation[19]. After moistening the swabs with sterile normal saline, surplus was eliminated by pushing the swab stick against the tube's inner surface[20]. Individual moistened sterile cotton swabs were used to swab the door and bed-side handles. This was accomplished by swabbing the up/down surfaces and left/right surfaces, and then swab stick was recapped. After being properly labeled with the number, place, and date, the specimens were delivered within four hours of collection to the Microbiology Laboratory at Nnamdi Azikiwe University Teaching Hospital Nnewi, where they were examined between twenty-four hours.

2.8 Sample Processing and Determination of Pathogen

The swab samples of the door and bedside handles were inoculated onto Cetrimide agar, specific for isolating *Pseudomonas aeruginosa* (using conventional surface streaking). For twenty-four hours, the infected petri dishes were incubated at 37°C. The plates were examined for bacterial growth.

2.9 Preparation of Working Media

2.9.1 Cetrimide Agar

This is an enriched medium. It is differential as well as selective. It favors the development of *Pseudomonas aeruginosa* in particular, producing pyocyanin, a blue pigment and siderophore that differs greatly from the medium's initial color. Cetrimide agar was prepared following the manufacturers' instruction.

2.9.2 Nutrient Agar

This is a general medium for most bacteria. It was made in compliance with the guidelines provided by the company that made it.

2.9.3 Mueller Hinton Agar (MHA)

This is also a general medium but best for antibiotic susceptibility testing (AST). It was made in compliance with the guidelines provided by the company that made it.

2.9.4 Identification of the isolate

Microscopic, macroscopic, and cultural analyses were performed. The physical traits of the colonies—sizes, coloration, elevation, consistency, and odor—were used to choose them. Both standard biochemical assays and Gram staining were carried out[20].

2.9.5 Gram Staining Technique

Gram staining procedure was carried out to differentiate isolates into Gram-positive and Gram-negative organisms. A small amount of the organism selected from a growing colony of 18–24 hours old pure culture was emulsified into a drop of sterile distilled water on a grease-free slide to create thin smears. After letting the smears air dry, they were heat-fixed by passing them close to a flame. After carefully positioning the slides on the staining rack, they were submerged in crystal violet, the principal stain, for 30 to 60 seconds. For 30 seconds, Lugol's iodine (a mordant) was added. Tap water was used to gently rinse the stains. To act as a decolorizer, acetone was administered and removed right away. After applying the neutral red counter stain and letting it sit for 30 to 60 seconds, the area was cleaned with tap water once more and left to dry. After that, the smears were inspected for potential gram-negative diplococci under a microscope using an xl00 oil immersion objective lens.

2.9.6 Motility Test

The isolates were tested for motility. A drop of regular saline was applied on a sterile, grease-free slide as part of the wet preparation process. A sterile applicator stick was then spread over the saline to select a colony of the organism. With the condenser iris properly closed, a sanitized cover slip was put over the smear and examined under a microscope with 10x and 40x scopes. The organism's motility was demonstrated by the bacterium particles' directed movement.

2.10 Biochemical Test

2.10.1 Oxidase Test

A filter paper was placed on a sterile petri dish to test the isolates for the production of the oxidase enzyme. A colony of the cultivated organism was selected using a sterile applicator stick and smeared in the oxidase reagent on the filter paper after a drop of the freshly made oxidase reagent was applied to the paper using a sterile Pasteur pipette. A change to deep purple color was observed, showing a positive reaction.

2.10.2 Indole Production

The isolates were tested for indole production by inoculation into peptone water in sterile bijou bottles and incubated overnight at 37^0C. Three drops of Kovacs reagent were added to each overnight broth. There was no red ring indicating indole production, so it's a negative reaction.

2.10.3 Citrate Utilization

The citrate agar was made in compliance with the guidelines provided by the company that made it; autoclaved and then poured into various test tubes

and bijou bottles, slanted on a sterile surface and allowed to cool and gel. A colony of the cultivated organism was selected using a sterile wire loop, and the tubes and bijou bottles were aseptically streaked and stabbed at the butt. Overnight incubation at 37^0C °C showed a change of colour from green to blue, indicating citrate production and a positive result.

2.10.4 Urease Test

The urea agar was made in compliance with the guidelines provided by the company that made it and poured into various test tubes and bijou bottles. They were autoclaved, placed on a sterile surface and allowed to cool and gel. Using a sterile wire loop, a group of the cultivated organism was selected, aseptically punctured at the tubes' and bijou bottles' butts, and incubated at 370C for the whole night. There was no change of colour from yellow to pink, indicative of a negative result.

2.10.5 Catalase Test

Three drops of hydrogen peroxide (H2O2) were aseptically placed on a sterile, grease-free slide using a sterile Pasteur pipette to test the isolates for catalase production. A sterile applicator stick was used to select a colony of the cultivated organism, which was then introduced into the hydrogen peroxide. There were visible air bubbles, which suggested the formation of catalase, and *P. aeruginosa* was highly suspected.

2.10.6 Sterility Test

Sterility test was done for the Nutrient, MHA, and Cetrimide agar (stored at 4°C) to ascertain for any contamination. Nutrient, MHA, and Cetrimide agar sterility tests were done by picking at random, one from each batch of the prepared agar, and incubating overnight at 37°C. Any visible growth indicates contamination, and that batch of agar was discarded, and new agar was prepared.

2.10.7 Turbidity Standard for Inoculum Preparation

Using a barium sulphate (BaSO4) turbidity standard, which is comparable to a 0.5 McFarland standard ($1–2×10^8$ cfu/mL), the inoculum density for a susceptibility test was standardized[21].

2.10.8 Standardization of Test Organisms

The test organism's overnight agar culture provided the inoculum. At least three to five well-isolated colonies with the same shape were taken from an agar plate culture to provide the inoculum for the susceptibility test. A sterile wire loop was used to contact the top of each colony. The growth was then moved into a tube with three milliliters of sterile normal saline and vigorously mixed to create a smooth suspension. By comparing the inoculum tube with the 0.5 McFarland standard tube, the inoculum's density was then corrected using sterile saline to 0.5 McFarland standard. This produced a suspension with around $1–2×10^8$cfu/mL of the test organisms. Within fifteen minutes, the standardized test organism suspensions were put to use.

2.11 Disc Diffusion Susceptibility Test

2.11.1 Modified Kirby-Bauer Disc Diffusion Technique

A standardized inoculum suspension of the bacteria was prepared. In order to reduce the quantity of condensation that happens when warm air comes into touch with a cold surface, nutrient agar and disc bottles were allowed to get to room temperature by standing for one to two hours before the inoculum was standardized. To remove extra inoculum from a sterile cotton swab, it was dipped into the standardized inoculum solution, turned many times, and pushed firmly against the tube's inner wall above the fluid level. The whole sterile agar surface was streaked with the swab to inoculate the dried surface of a nutritional agar plate. To guarantee a uniform dispersion of inoculum, streaking was done twice more while rotating the plate at a temperature of around 60°C each time. The agar's rim was swabbed as a last step. The prepared discs were applied after the lid was left open for 3 to 5 minutes, but no more than 15 minutes, to absorb any excess surface moisture.

The commercially prepared discs; Nitrofurantoin (300µg), Augmentin (30µg), Cephalosporins {Ceftazidime (30µg), Cefixime (5µg), Cefuroxime (30 µg)}, Aminoglycosides {Gentamycin (10µg)} and Fluoroquinolone {Ciprofloxacin (5µg), Ofloxacin 30µg} was applied appropriately to the infected agar plate's surface. Using sterile forceps, each disc was positioned separately before being gently pushed down into the agar. A disc won't move once it makes contact with the agar surface because the key components in the disc start to diffuse right once. The discs were equally spaced, with a maximum distance of 24 mm between centers. Within an hour of the discs being applied, the plates were inverted and put in an incubator that was set at 35°C.

Bacterial growth surrounding each disk was seen during an overnight incubation. A distinct region of "no growth" was seen surrounding that specific disc, which is known as the zone of inhibition, indicating that the test isolate is sensitive to that specific antibiotic.

In line with NCCLS (National Committee for Clinical Laboratory Standards), the zone diameter was measured using a ruler to the closest whole millimeter (mm)[22] and compared to a standard interpretation chart used to classify the isolate as susceptible, intermediately susceptible, or resistant. In cases when zones of neighboring discs overlap, the zone diameter was calculated by multiplying the radius of the zone by two (2) and measuring the distance from the disk's center to a point on the zone's perimeter where a clear edge was present.

2.11.2 Detection of MBL (Metallo-Beta-lactamase) producing strain of the isolated P.aeruginosa

MBL production was determined using the Total MBL Confirm Kit from ROSCO Diagnostica (ROSCO Diagnostica A/S, Taastrupgaardsvej, Denmark); by the disc diffusion method using EDTA (Ethylene diamine tetra-acetic acid)-impregnated imipenem and DPA-(Dipicolinic acid) impregnated imipenem discs. The ROSCO Diagnostica total MBL confirm kit has a sensitivity of 97.7% and a specificity of 100%.[23]. According to the kit's instructions; only Ceftazidime-resistant isolates should be tested to avoid obtaining false MBL-positive isolates (if Ceftazidime sensitive).

2.12 Interpretation of Results

In order to understand the data, the inhibition zones of the various pills were compared. Imipenem 10ug (IMI10) inhibition zones were examined and contrasted with Imipenem + DPA (IM+DP) and Imipenem + EDTA (IM10E). IM + DP and IMI10 ≥ 5 mm and/or IMI10E and IMI10 ≥ 8 mm are the zones that vary, indicating that the isolate generates a metallo-beta-lactamase. There is synergism if the IMI10 inhibition zone is absent (9 mm), the IM+DP inhibition zone is ≥ 12 mm, and the IM10E inhibition zone is ≥ 13 mm. This indicates that Imipenem + DPA (but not Meropenem + DPA) can be used to detect MBLs in *Pseudomonas aeruginosa* with a 99% sensitivity and a 95% specificity. MBLs in Enterobacteriaceae should be detected using meropenems with DPA.

2.13 Quality Control

Pseudomonas aeruginosa from a hospital was employed as a positive control. A hospital strain of Escherichia coli (E. coli) served as the negative control. The best therapeutic choices for *P. aeruginosa* that produces MBL, according to data extrapolated from in vitro trials, are polymyxin B or colistin (DPA)[24].

2.14 Statistical analysis

Data analysis was conducted using the Statistical Package for the Social Sciences (SPSS) version 21.

3. Results

A total of 125 swab samples comprising of laboratory and ward door handles; as well as bed side handles of hospitalized patients randomly selected in Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, were analyzed to determine the presence of *Pseudomonas aeruginosa*and the Metallo Beta-lactamase (MBL) strains phenotypically. The control organism and 7 others were isolated as *Pseudomonas aeruginosa* bringing the total number of *Pseudomonas aeruginosa* isolatesin the study to 8. The antibiotic susceptibility to different antibiotics which include: Nitrofurantoin (NIT), Augmentin (AUG), Ceftazidime (CAZ), Cefixime (CXM), Cefuroxime (CRX), Gentamycin (GEN) and Ciprofloxacin (CPR), Ofloxacin (OFL)were tested against the bacteria isolated. The ceftazidime resistant strains were then particularly tested for MBL according to the instruction of the diagnostic kit. Out of the 8 organisms; 4 were ceftazidime resistant and so further testing with Imipenem 10ug, coded **IMI10;** Imipenem 10ug + Dipicolinic acid, coded **IM+DP** and Imipenem 10ug + EDTA, coded **IM10E** were used to actually confirm if these four (4) isolates are MBL producers by their synergistic activity to inhibit production of the enzyme or show resistance to IMI10. Three were MBL producers because the synergistic activity of the antibiotics was able to inhibit enzyme production, and so a clear zone of inhibition was seen. One is an MBL producer because it showed resistance to imipinem10ug.

Table 1. *Number of Pseudomonas aeruginosaisolated*

Fomites used in study	Total Samples Taken	Total number of *Pseudomonas aeruginosa* isolated	Prevalence (%)
Lab door handle	37	0	0
Ward door handle	7	2	25.0
Patient's bed-side handle	81	5	62.5
Control	1	1	12.5
Total	126	8	100%

The total number of *Pseudomonas aeruginosa* isolated was 8, of which 2 were isolated from door handles of wards; while 5 were isolated from bedside handles of patients, then the control, giving it a prevalence of 25%, 62.5% and 12.5% respectively. None was isolated from the door handles of laboratories (Table 1).

Out of 2 *Pseudomonas aeruginosa* isolated from door handles of wards; only one(1) was Ceftazidime resistant; out of 5 isolated from bed side handles of patients; two (2) were Ceftazidime resistant, then the control was also Ceftazidime resistant; giving it a prevalence of 25%, 50% and 25% respectively. So out of eight (8) isolated *P. aeruginosa;* four (50%) are Ceftazidime resistant. This shows that *Pseudomonas aeruginosa* has a chi-square of 23.08 and a p-value of 0.00, which is termed significant when compared to the control significance value of ($p<0.05$) (Table 2).

Table 2. *Number of Ceftazidime-Resistant Isolates*

Fomites used in study; (positive for *Pseudomonas aeruginosa)*	Number of *Pseudomonas* isolates	Isolates with Ceftazidime resistance.	Frequency	Prevalence (%)
Ward door handle	2	1	1	25.0
Patient's bedside handle	5	2	2	50.0
Control	1	1	1	25.0
Total	8	4	4	100%

Table 3. *Constituents of the MBL- detection kit used*

Name of Antibiotic	Constituent and concentration(ug)	Manufacture date	Expiration date
Imipenem	Imipenem (10ug)	12-09-2017	11-09-2021
Imipenem and Dipicolinic acid	Imipenem (10ug) Dipicolinic acid (5ug)	12-09-2017	11-09-2021
Imipenem and EDTA	Imipenem (10ug) EDTA (3.75ug)	12-09-2017	11-09-2021

All the Ceftazidime-resistant *Pseudomonas aeruginosa* are MBL-producers through different mechanisms. The strain isolated from a ward door handle showed MBL production by being resistant to Imipenem, giving a prevalence of 25%. Two (2) other isolates from the patient's bedside handles showed MBL-production by growth inhibition, giving a prevalence of 50% and the control (which is also a hospital strain), showed MBL-production by growth inhibition, giving a prevalence of 25% too. This showed that MBL-producing *Pseudomonas aeruginosa* has a chi-square of 39.02 and a p-value of 0.00, which is termed significant when compared to the control significance value of ($p<0.05$) (Table 4).

Table 4. *Result of total number of MBL - producing Isolates*

Fomites used in study; (positive for *Pseudomonas aeruginosa)*	Isolates with Ceftazidime resistance	MBL- Production		Frequency	Prevalence (%)
		By Resistance to Imipenem	By Growth-Inhibition		
Ward door handle	1	1	0	1	25.0
Patient's bedside handle	2	0	2	2	50.0
Control	1	0	1	1	25.0
Total	4	1	3	4	100%

The Male Surgical Ward (MSW) has the highest prevalence of *Pseudomonas aeruginosa* (57.14%), followed by the Children ward (28.57%) and then the Female Surgical Ward (FSW) (14.29%). For MBL-producing *Pseudomonas aeruginosa*, MSW had the highest prevalence (66.67%); followed by the Female Surgical Ward (33.33%). There was no MBL-producing *Pseudomonas aeruginosa* isolated from the children's ward (Table 5).

The different zones of inhibition in mm for all the MBL-producing strains isolated. Imipenem chelated with EDTA shows the highest zone of inhibition (29mm); followed by Imipenem chelated with Dipicolinic acid (28mm). Using Imipenem alone can lead to little or no growth inhibition at all (Figure 1).

Table 5. *Prevalence of Pseudomonas aeruginosa and MBL-producing Pseudomonas aeruginosa in the sampled wards.*

Wards	Number of *P.aeruginosa* isolates	Site of isolation	Prevalence (%)	Number of MBL-producing*P.aeruginosa*	Prevalence (%)
Male surgical Ward (MSW)	4	Bedside handles	57.14	2	66.67
Female surgical Ward (FSW)	1	Door handle	14.29	1	33.33
Children ward(PAED EXT, CHER, PAED SURG, PAED WARD)	2	Door handle and Bedside handle	28.57	0	0
Total	**7**		**100**	**3**	**100**

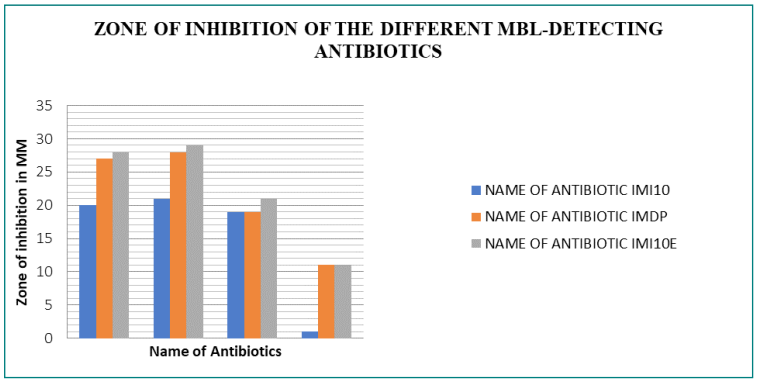

Figure 1. *Zone of inhibition of the antibiotics used for MBL detection*

4. Discussion

Pseudomonas aeruginosa is a key opportunistic pathogen and a major source of hospital-acquired infections. Metallo-β-lactamases (MBLs) are crucial mechanisms behind Carbapenem resistance in *P. aeruginosa* isolates[12]. Human hands significantly contribute to the spread and cross-contamination of microorganisms on environmental surfaces, such as door handles and bedside rails—common contact points that can facilitate pathogen transfer. This means patients or healthy healthcare workers can become secondary infections or unknowingly carry resistant strains, making treatment more challenging. Pathogens on fomites can survive from hours up to months, even after routine cleaning[25]. This study found high contamination levels—62.5%—on patient bedside handles. Environmental factors like humidity and moisture influence microbial transfer rates. Ghonaim and Nada[26] isolated more resistant *P. aeruginosa* strains from hospital fomites than from patient or staff samples, aligning with this study's findings. Surface contamination remains a public health concern, highlighting the need for strict policies to reduce hospital surface bio-contamination.

Out of 126 samples, only 8 (6.35%) *P. aeruginosa* isolates were identified, consistent with Abaza et al. (2017), who found 11.3% in 1583 clinical samples. All 30 tested *P. aeruginosa* isolates in that study were MBL-positive, matching this study's results, where all ceftazidime-resistant isolates were MBL producers.

The antibiotic susceptibility patterns helped determine MBL prevalence phenotypically. In Nnamdi Azikiwe University Teaching Hospital, *P. aeruginosa* was most frequently isolated from bedside handles (62.5%), followed by ward door handles (25%), and control strains (12.5%). MBL-producing strains were isolated from bedside handles (50%), ward door handles (25%), and control strains (25%). These findings somewhat align with Olise and Simon-Oke's study, which reported higher contamination at door knobs (23%) and other surfaces. In this study, the pediatric ward had the second-highest prevalence (28.57%), contrasting with Olise and Simon-Oke[27], where children's wards showed only 7.5%.

The Male Surgical Ward (MSW) showed the highest presence of *P. aeruginosa* (57.14%) and MBL producers (66.67%), differing from Olise and Simon-Oke's[27] findings, where MSW ranked lower. Variations likely stem from this study focusing solely on *P. aeruginosa*. The Female Surgical Ward (FSW) ranked third, consistent with their study's high contamination rates.

In this study, MSW had the highest prevalence of both P. *aeruginosa* (57.14%) and MBL-producing *P. aeruginosa* (66.67%). This agrees with the findings of a study by Marra et al.[24], which showed that fifty-seven

percent of patients infected with MBL-producing *P. aeruginosa* are males. There is no known cause for this. Also, in this study, *P. aeruginosa* isolated from children's ward recorded the second highest prevalence (28.57%). This concurs with a study by Simon et al.[28] which showed outbreaks of *Pseudomonas aeruginosa* in pediatric patients. These outbreaks are often caused by *P. aeruginosa's* persistence in environmental vectors and reservoirs (tap water, medical equipment, and fomites) or by specific violations of fundamental hygiene measures.

In Owliaet al.[29], 73.44% of the isolates were resistant to ceftazidime; this study showed a 50% resistance; and this may be because of the slight disparity in the sample number (128, this study used 125). In that same study, out of the 73.44% of *P. aeruginosa* that were resistant to ceftazidime, 53.2% of the isolates were MBL positive; this is quite contrasting to the result of this particular study, as 100% of the ceftazidime-resistant isolates were MBL-producing. Again, this could be a query on numbers because just 4 out of 8 isolates were ceftazidime resistant, meanwhile 50 out of 94 isolates were ceftazidime resistant in that study. The disparity in the result is not surprising, though, because according to a study by Hong et al.[30] there is a high prevalence of the MBL-gene in that part of the world. Owliaet al.[29] conducted their study in Iran.

The current study's findings demonstrated that a specific strain that was isolated from the FSW door handle exhibited imipenem resistance. This supports the findings of the study by Pitout et al.[31], which show that all isolates resistant to carbapenem can produce metallo-β-lactamase (MBL). In another investigation, Farhan et al.[32] discovered that 52.3% (eleven out of twenty-one) of carbapenem-resistant isolates produced MBL. This is contradictory to the above study by Pitout et al.[31] and further studies are needed, as only one imipenem-resistant organism was isolated from this study and following the procedure of the diagnostic tool used in the present study.

The exact procedure used for this study was used in a study conducted by Saderi et al.[33], and so confirmed that there is a high possibility for Imipenem-resistant strains to produce the MBL enzyme as seen in this study. 65 out of 69 imipenem-resistant strains had MBL activity. The disparity in the previous study by Farhan et al.[32] may be as a result of the slight difference in drug class between imipenem and carbapenem, but both studies are in agreement with the findings in this study that administering these antibiotics with EDTA can still produce a bactericidal effect on the MBL-producing *P. aeruginosa*. Behera et al.[34] concurs with this study in the synergy of Imipenem and EDTA to detect MBL-producing *P. aeruginosa*, as well as Imipenem inhibition to detect the same.

In research by Khosravi and Mihani[35], eight (19.51%) of the 41 imipenem-non-susceptible isolates found seemed to generate MBL, as determined by Etest and verified by PCR assay. This calls for further molecular studies on this work to actually detect MBL genes in the imipenem-resistant isolate to confirm MBL-production. This current study revealed that door handles of wards and patient bedside handles are contaminated by different strains of *P. aeruginosa*, including resistant strains. The hands of individuals are a deadly weapon. The cycle continues as contaminated and inadequately cleaned hands contaminate bedside and door handles, which contaminate hands and ultimately lead to infection persistence in the hospital environment. Rapid identification of MBL-producing isolates and the implementation of suitable infection control strategies are essential to stop the spread of infections caused by these organisms, given their high prevalence and clinical significance. It is concerning because MBL producers are spreading so quickly, and this calls for the use of both monitoring studies and appropriate and prudent antibiotic selection, particularly with regard to imipenems.

5. Conclusion

Although this study involves only a limited number of sampled populations, it confirms that nosocomial infections continue to persist in healthcare settings, as shown in similar research. Fomites are demonstrated to act as reservoirs for pathogenic microorganisms, evidenced by the isolation of a leading pathogen and its resistant strains from fomite surfaces. Despite not being a common focus, isolating resistant strains from fomites is crucial, as this study indicates that fomites can serve as transmission vehicles for resistant nosocomial organisms within healthcare environments. Therefore, thorough hand washing, disinfection, and careful contact control procedures are essential to reduce the spread of bacteria like P. aeruginosa and resistant strains. Additionally, before treatment, antimicrobial susceptibility testing (AST) should be performed to identify the appropriate antibiotic, preventing further resistance development and ensuring effective treatment.

6. References

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